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PROTEIN POLYMORPHISM OF THE HYBRIDIZING SEASTARS *ASTERIAS FORBESI* AND *ASTERIAS VULGARIS* AND IMPLICATIONS FOR THEIR EVOLUTION

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We wish to present an initial comparison of biochemical and morphological data for the naturally hybridizing seastars *Asterias forbesi* (Desor) and *A. vulgaris* Verrill. The similarity in morphology between these two extremely abundant New England seastars has been noted repeatedly in the past hundred years (Verrill, 1866; Coe, 1912 and Aldrich, 1956). Nonetheless, they cannot be regarded as typical "sibling species" since 7 prominent characters of the external phenotype are characteristic of each form (Table I). In sampling local populations, no single characteristic appears to be completely reliable. However, a comparison of the complete list of characteristics usually allows definite assignment of a specimen to one taxon or the other.

Occasionally specimens are encountered which are clear morphological intermediates between the two species. Anecdotal information on the existence of hybrids has been reported (Clark, 1904; Sanchez, in Edmondson, 1966, page 41). In laboratory cultures, eggs of each species are able to be fertilized by sperm of the other species (Ernst, 1967). Hybrid formation evidently does not lead to massive introgression since hybrids have been found only at localities where typical "good" representatives also occur. That there is some introgression is evidenced by the lack of complete reliability of any one morphological characteristic. The specific mechanism evidently blocking introgression is unknown. The claim has been made that "introgressed individuals are normally eliminated by natural selection" (Mayr, 1963, page 132), although limited experimental evidence suggests the opposite (Lewontin and Birch, 1966).

Although *Asterias forbesi* ranges from the Gulf of Mexico to Maine, (Fig. 1), from intertidal to 100 m depths, north of Cape Cod it chiefly occurs in warmer waters of inshore bays, and is increasingly rarer. Its place is taken by *A. vulgaris* which is found from Cape Hatteras to Labrador, and south of Cape Cod it is limited to the deeper, colder offshore waters, to 650 m depths. The two species are sympatric in offshore areas south of Cape Cod and in a few harbors north of the Cape.

Materials and Methods

Specimens of *Asterias forbesi* were collected from Woods Hole, Massachusetts, on the rocks in front of the Marine Biological Laboratory. Specimens of *A. vulgaris* were obtained at the east end of the Cape Cod Canal at Cape Cod Bay (Fig. 1). After starving individuals for three to ten days after collection, the hepatopancreas

TABLE 1

Morphological characteristics used to distinguish Asterias forbesi *and* A. vulgaris *(from Verrill, 1866; Coe, 1921; and Aldrich, 1956)*

Character	*Asterias forbesi*	*Asterias vulgaris*
Shape of ray	Slender base, blunt tip	Thick base, tapered tip
Endoskeleton	Firm, formed of interlocking plates	Flaccid, formed of narrow, bar-like plates with large inter-spaces
Abactinal surface of the ray	Arched	Flattened
Abactinal spines	Scattered	Concentrated into a single median row on each ray
Major pedicellaria of adambulacral spines	Broad, with rounded tip	Elongate with pointed tip
Color of madreporite	Pale orange to red-orange	Cream
Optical peduncle	Eye unstalked	Eye born on a fleshy stalk

was removed from one or more of the starfish arms of a single individual and placed in a 1 ml centrifuge tube for grinding with a plastic rod rotated in an electric eraser. After centrifugation at about 21,000 rpm for three minutes, 10–20 μl of supernatant was placed in one of 24 slots of a vertical acrylamide electrophoresis unit (Aardvark Industries; Lombard, Illinois). Electrophoresis (7.5% gel) was usually performed for 2½ hours at 400 V (30–140 mAmp). In addition to analyzing several individuals of each species of *Asterias* on a single gel, at least one individual of the sea stars *Leptasterias tenera* and *Henricia sanguinolenta* was analyzed for comparison in the same way on each gel for each enzyme system.

Thirty-three protein systems were tested for suitability of which 9 yielded distinct band patterns (Fig. 2). Data from 19 to 72 individuals (usually 24–36) were used to characterize each band pattern. Procedures for staining gels were those reported by Shaw and Prasad (1970) for sorbitol dehydrogenase (SDH), phosphoglucose isomerase (PGI), and hexokinase (HK); by Hubby and Lewontin (1966) for leucine amino peptidase (LAP, with the minor change that Fast Black K is added at the same time as the substrate), general protein (GP, using amino black and coomassie blue), and malate dehydrogenase (MDH, at pH 7.6, 50 mg NAD, 50 mg NBT, 150 mg sodium salt of malic acid); by Brewer (1970) for fructokinase (FK); by Gooch and Schopf (1970) for esterase (E), and tetrazolium oxidase (TO); and by Yang as cited in Selander, Smith, Yang, Johnson and Gentry (1971) for glutamate oxalate transaminase (GOT). "Nothing" kinase (NK) and "Nothing" dehydrogenase (ND) develop using tetrazolium in the absence of added substrate.

RESULTS

Data are given only for individuals whose external morphology appeared typical of *Asterias forbesi* or *A. vulgaris*. Protein bands in the 9 protein systems are attributable to 27 band systems in *A. forbesi* and 26 in *A. vulgaris*, tentatively

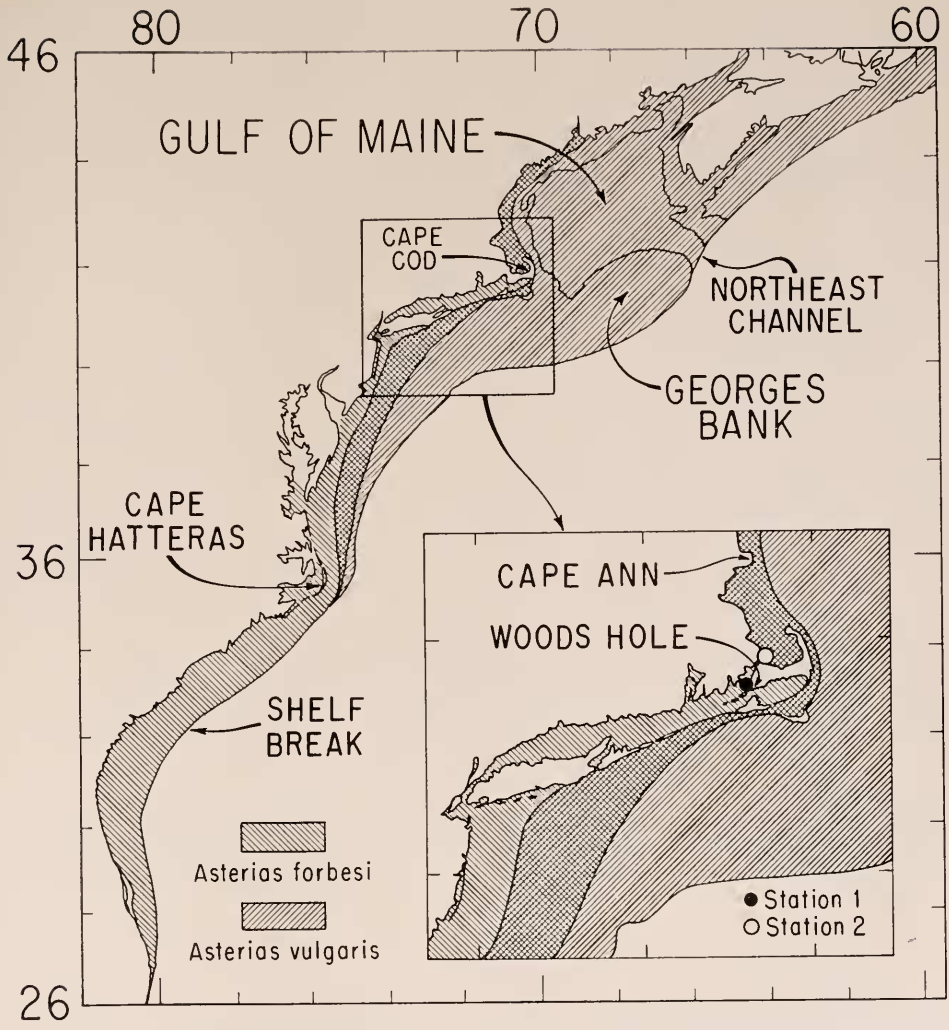

FIGURE 1. Chart of Atlantic coast to show the distribution and probable overlap in range of the seastars *Asterias forbesi* and *A. vulgaris* (from Clark, 1904; Gray, Downey and Cerame-Vivas, 1968; and collections of L. S. Murphy); *A. forbesi* from station 1, and *A. vulgaris* from station 2.

equated with 27 and 26 gene loci. As is customarily done, we assume that the presumptive gene loci are a representative sample of structural loci in the genome of each species of *Asterias*. The biochemical data of this paper are restricted to a comparison of enzymes and other proteins of these two closely related species, although we note that in general the electrophoresis patterns of *Asterias* spp. were quite distinct from those recorded for *Henricia* and *Leptasterias*.

Allele frequencies for each locus (Table II) were considered according to the procedure of Nei (1972) to obtain an estimate of the degree of genetic similarity be-

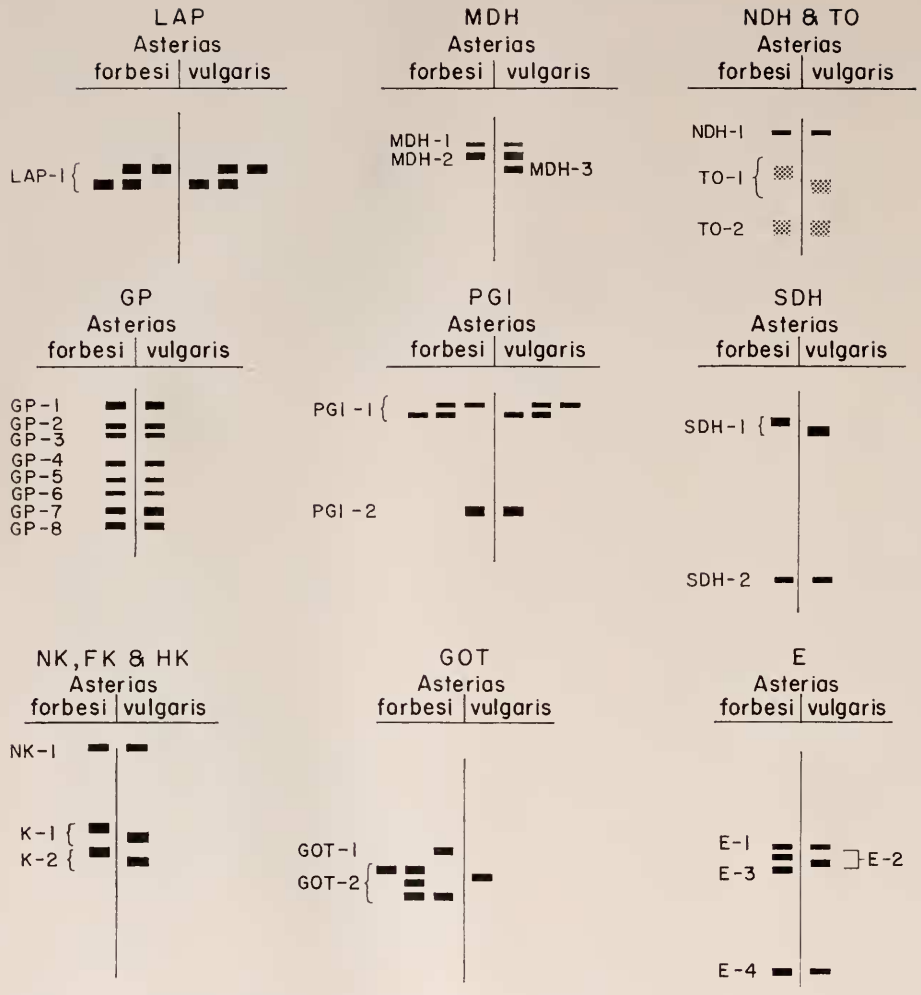

FIGURE 2. Diagram indicating band patterns and mobility relationships of protein zones of the seastars *Asterias forbesi* and *A. vulgaris*. For each protein the origin is at the top and the direction of mobility toward the bottom. Mobilities are given for typical runs of 2½ hours at 400 V and about 80 mAmp. Abbreviations stand for sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH), tetrazolium oxidase (TO), general protein (GP), phosphoglucose isomerase (PGI), leucine amino peptidase (LAP), fructokinase (FK), hexokinase (HK), glutamate oxalate transaminase (GOT), Esterase (E), "nothing" dehydrogenase (NDH), and "nothing" kinase (NK). In addition to the systems indicated, future studies may reveal polymorphic systems between the origin and GP-1, and between GP-3 and GP-4. In PGI, a definite pink band develops just below PG-1. In SDH a narrow pink band occurs in a pinkish zone between SDH-1 and SDH-2. In MDH an uninterpretable but darkly staining band system exists below the TO-1 zone. MDH-1 is inhibited on gels to which has been added 50 mg HCN per 100 ml staining solution.

tween *Asterias forbesi* and *A. vulgaris*. On the average, each species shares 67% of its genes in common with the other species, for this sampling of gene loci. The genetic distance (D) is 0.397 which is the accumulated number of allelic differences per locus.

TABLE 11

Allele frequencies for 27 loci in Asterias forbesi *and* A. vulgaris. *Abbreviations as in text. Band patterns in Figure 2*

Locus	Allele	*Asterias forbesi*	*Asterias vulgaris*	Locus	Allele	*Asterias forbesi*	*Asterias vulgaris*
SDH-1	a	1.0	0.0	PGI-1	a	0.63	0.84
	b	0.0	1.0		b	0.37	0.16
GP-1	a	1.0	1.0	PGI-2	a	1.0	1.0
GP-2	a	1.0	1.0	GOT-1	a	1.0	0.0
GP-3	a	1.0	1.0	GOT-2	a	0.89	0.0
GP-4	a	1.0	1.0		b	0.0	1.0
GP-5	a	1.0	1.0		c	0.11	0.0
GP-6	a	1.0	1.0	LAP-1	a	0.66	0.79
GP-7	a	1.0	1.0		b	0.34	0.21
GP-8	a	1.0	1.0	E-1	a	1.0	1.0
NK-1	a	1.0	1.0	E-2	a	1.0	0.0
K-1	a	1.0	0.0		b	0.0	1.0
	b	0.0	1.0	E-3	a	1.0	0.0
K-2	a	1.0	0.0	E-4	a	1.0	1.0
	b	0.0	1.0				
MDH-1	a	1.0	1.0				
MDH-2	a	1.0	1.0				
MDH-3	a	0.0	1.0				
TO-1	a	1.0	0.0				
	b	0.0	1.0				
TO-2	a	1.0	1.0				
NDH-1	a	1.0	1.0				

Considering each species separately, for *Asterias forbesi* 11% of the loci (27) are polymorphic (at least 2 alleles with a frequency of more than 5%), and the heterozygosity per individual is 2.1% (calculated as in Lewontin and Hubby, 1966). For *A. vulgaris,* 7.7% of the loci (26) are polymorphic, and the heterozygosity per individual is 1.1%.

DISCUSSION

A figure of 67% genic similarity in *Asterias forbesi* and *A. vulgaris* is within the range of values for studies of sibling species. In an investigation of nine triads of *Drosophila* (a sibling species pair plus one close relative), "As an overall average, members of a sibling pair have 50% of their proteins in common, while nonsibling members of a triad share only 18% of their proteins" (Hubby and Throckmorton, 1968, page 198). The percentage identity of proteins between sibling species in their study ranged from 22.5% to 85.7%. Comparing seastars and fruit flies, similar degrees of genetic differentiation result in quite different degrees of morphologic differentiation.

Other data comparing complexes of related species support the general conclusion that closely related species share a significant proportion of their loci in common. These include other species of *Drosophila* (Ayala, Mourão, Pérez-Salas, Richmond and Dobzhansky, 1970; Yang, Wheeler and Bock, 1972) and small mammals (Selander, Hunt and Yang, 1969; Johnson and Selander, 1971; Johnson, Selander, Smith and Kim, 1972). *Asterias forbesi* and *A. vulgaris* are interpreted

as another example of "semispecies" (Mayr, 1963, page 118), that is, closely related species lacking complete reproductive isolation and sharing a significant proportion of their genes. This is the first case of semispecies in marine invertebrates for which this measure of genetic similarity is presented.

Of the many mechanisms which could result in reproductive isolation, the two which appear most likely are temperature tolerance and temporal isolation of spawning time. As charted 60 years ago, temperature now appears to be the limiting factor in the distribution of these species (Sumner, Osburn and Cole, 1913, page 113, charts 48, and 49). However, we are not aware of colaborative physiological studies of temperature tolerance on the reproduction or survival of these two species. Data exist on spawning time, but are inconsistent. Coe (1912) reported *A. forbesi* spawning in Long Island Sound in June. Loosanoff (1961) stated that spawning occurs in these same waters in July, but with local variation. *A. vulgaris* spawns in June and early July in Woods Hole (Field, 1892), and in late May and early June in the Gulf of St. Lawrence (Smith, 1940). All reports indicate a wide range of spawning times, with ripe inviduals found in the autumn.

How did *Asterias forbesi* and *A. vulgaris* originate? If glacial ice were to melt completely, sea level would rise about 70 m, submerging eastern Massachusetts (Emery, 1967a; 1967b). In addition, prior to the glaciations, glacial sediments, about 70 m on Georges Bank (Uchupi, 1968, 1970), would not have been deposited. Thus, at least in the early Pleistocene, species of *Asterias* and other shallow marine invertebrates would have freely ranged north and south of the present Cape. With subsequent sea level lowering, species ranges would have been split. At 15,000 years ago, sea level was approximately 130 m below its present level (Milliman and Emery, 1968; Emery, Niino and Sullivan, 1971). As sea level rose, arctic waters advanced into the Gulf of Maine, just as the sea today advances into fiords of Norway. Southern areas were receiving warm water from the Gulf Stream circulation. Perhaps 6000 to 7000 years ago some interbreeding would have been possible as the sill depth between the Gulf of Maine and the region to the south may have been breached. Presumably sufficient ecologic differentiation had occurred by then so that subsequent evolution has been largely independent. The alternative explanation—that *A forbesi* and *A. vulgaris* developed sympatrically—is of course possible. However, because of the reasonable presumed influence of glaciation in altering sea level and dividing species ranges, a sympatric origin seems to be less plausible, or at least to demand more stringent assumptions.

If the evolution of the northern *Asterias vulgaris* and the southern *A. forbesi* progressed as outlined above, then other pairs of species with similar origins are to be expected. E. L. Bousfield has recently (1973) published a detailed monograph of the amphipods of New England to depths of 30 m. According to our analysis of the distributional records in his monograph, 96 species have one boundary of their distribution associated with Cape Cod. We found 9 pairs of gemminate species, or approximately 20% of the amphipods of shallow waters. For other groups, Sumner, Osburn and Cole (1913) have a section entitled "Comparative Distributions of Closely Related Species" in which are cited records of possible equivalent types of species for hydroids, crabs, and clams. More recently documented examples may include mysids (Wigley and Burns, 1971), ostracods (Hazel, 1970), and ectoprocts (Osburn, 1933; Maturo, 1968; collections of T. J. M. Schopf).

These species pairs appear to be marine examples of the phenomenon well known on land of the influence of glaciation leading to geographic speciation, for which even the occurrence of hybrids is well established (Rand, 1948; Mayr, 1963, pages 369–372; Blair, 1951; Deevey, 1949, pages 1335–1338). Mayr (1963, page 372) states that "Most hybrid zones in the temperate region are the result of the fusion of populations expanding into the areas vacated by the retreating ice."

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Summary

The seastars *Asterias forbesi* and *A. vulgaris* share 67% of their genes in common (based on 27 loci). These species are normally easily characterized by 7 prominent phenotypic differences but naturally occurring hybrids are found in localities with typical adults of the two species.

A. forebesi and *A. vulgaris* are thought to have evolved during the mid to late Pleistocene as a result of a restriction in the range of a more widely distributed Miocene or early Pleistocene form due to lowering of sea level and the coincident emergence of a disrupting land barrier (Cape Cod-Georges Bank). At least one local population of the ancestral species evolved into the present cold water form (*A. vulgaris*) during selection in an arctic-fed Gulf of Maine. Coincidently, at least one other local population of the ancestral species evolved with selection in warmer, southern waters into the present shallow water, temperate form (*A. forbesi*).

If *A. forbesi* and *A. vulgaris* have been derived from a late Tertiary wide-ranging species which underwent geographic partitioning during the late Pleistocene, then both their present distribution and their lack of total reproductive isolation are understandable.

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